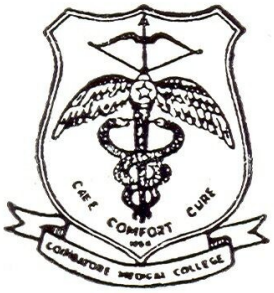
Evaluation of Multi Drug Resistant Pseudomonas Aeruginosa Isolates in Chronic Suppurative Otitis Media



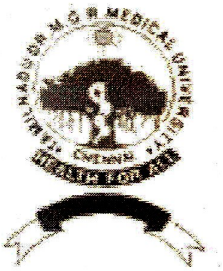
Dissertation submitted in

Partial fulfillment of the regulations for the award of

M.D. DEGREE

in

MICROBIOLOGY – BRANCH IV



The Tamilnadu

DR. M.G.R. MEDICAL UNIVERSITY

Chennai.

March 2007

DECLARATION

I DR P. SANKAR, hereby declare that the dissertation entitled **"Evaluation of Multi drug resistant pseudomonas aeruginosa isolates in chronic Suppurative otitis media"** to the Dr.M.G.R. medical university in partial fulfillment of the requirements for the award of M.D. Degree in microbiology in a record of original research work done by me during 2005-2006 under the super vision and guidance of Dr. R.K. Geetha professors and head of the Department of microbiology Coimbatore medical college Coimbatore – 14 and it has not formed the basis for the award of any degree / Diploma/ Association/ fellowship or other similar title to any candidate on any university.

Counter Signed Signature of Candidate

Head of the Department

CERTIFICATE

This is to certify that the dissertation, entitled **"Evaluation of multidrug resistant pseudomonas aeruginosa isolates in chronic Suppurative otitis media",** submitted to Dr. M.G.R. medical university, in partial fulfillment of the requirements for the award of M.D. Degree in microbiology is a record of original research work done by Dr. P. SANKAR during the period 2005 – 2006 of his study in Department of microbiology Coimbatore Medical College Coimbatore – 14 under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree/Diploma/Associateship / fellowship or other similar title to and candidate of any university.

Counter Signed Signature of guide

Dean.

ACKNOWLEDGEMENT

I extend my deepest gratitude to our Dean **Dr.T.P. KALANITI, Dean,** Coimbatore Medical College for allowing me to carry out this project.

I wish to articulate profound gratitude to **Dr. R. K. Geetha, MD** professor and head of the Department, Department of microbiology, Coimbatore Medical College, Coimbatore – 14. for giving me this opportunity, impeccable guidance, and Constant encouragement in the completion of this project.

I wish to express my sincere thanks to **Dr. Anbu. N Aravali, MD** Additional professor of microbiology department of microbiology, Coimbatore Medical College for his constant encourgement and support.

I owe my hearts felt guidance to **Dr. Kaliannan,** Head of the Department, Department of oto – Rhino laryngology microbiology Department Coimbatore Medical College, Coimbatore. For having allowed me to do the research work in ENT Department.

I take this opportunity to convey my thanks to **Dr. Rajendran, MD.,** Additional professor for his timely help and constant encouragement.

I wish to thank **Dr. Rajeshwari, MD.,** Additional Professor, Department of microbiology Coimbatore Medical College for her constant encouragement and timely help.

I wish to extend my special thanks to **Dr. M. Baskar, MD.,** Assistant professor Department of microbiology Coimbatore Medical College for guiding me through all my problems with constant encouragement and advice.

I wish to extend my special thanks to **Tmt. C. Gandhimathi** non-medical Assistant professor Department of microbiology for guiding me with full of encouragement and advice.

I acknowledge my indebtedness to all Technical Staff for their valuable help extended by them to me.

I thank all my dearest Friends and Classmates for there timely help and cooperation.

Last but not the least I thank my parents and family members, without their constant support I could not complete this research work.

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Introduction

Ear is the most important sensory organ concerned with the perception of hearing. Infections are the leading causes of deafness worldwide. (CSOM Burden of illness and management WHO 2004).

CSOM is a chronic Suppurative inflammation of mucoperiosteal layer of the middle ear cleft (Plate1). Incidence of CSOM higher in developing than developed countries particularly in India. The global burden of illness from CSOM involutes 65-330 million individuals with draining ears, 60% of whom 38-200 million suffer from significant hearing impairment CSOM accounts for 28000 deaths and a disease burden of over 2 million disability adjusted life years. Over 90% of the burden is borne by countries in south East Asia (WHO 2004) It shows the importance of controlling the infections effectively by for preventing the hearing loss all over the world. In developed countries the incidence of CSOM had come down to 0.04% of all cases of suppurative otitis media. In a rural area of India it was found to be 4.26%. Chronic suppurative otitis media is considered to be major problem in developing countries with relatively high mortality and morbidity (Glass cock et al; 1990). Pseudomonas aeruginosa is an opportunist pathogen that can infect almost any body site given the right predisposing conditions.

Pseudomonas aeruginosa is a significant pathogen among gram-negative organisms infecting ear (Brobby Gwetal ; 1987, Brook I et al., 1996). Infections caused by pseudomonas aeruginosa are often severe and life threatening and are difficult to treat because of the limited susceptibility

to anti microbial agents. (Carmeli; Y; et al ; 1999).

The problem of antibiotic resistance in Pseudomonas aeruginosa is on the increase. Accumulation of resistance after exposure to various antibiotics and cross-resistant among agents may result in multidrug resistant Pseudomonas aeruginosa.

Though chemical have been used against infections from 17th century the scientific era of anti microbial agents for combating infections started in the first decade of 20th century by paul Ehrlich based on the principle of

1. Selective Toxicity
2. Specific chemical relationship between infective agents and antibodies even though we could control the most of infections through antibiotics the development of drug resistance was noticed along side. Bacteria started developing multi drug resistance though genetic and non genetic mechanisms because of the selective pressure posed by the antibiotic on there survival.

In recent year Pseudomonas resistant to multi drugs including β-lactum antibiotics and extended spectrum of Cephalosporin is of great concern to ENT surgeons. Pseudomonas aeruginosa resists β lactum antibiotics by synthesising β lactamases. These enzymes inactivate cephalosporins by hydrolyzing the amide bond of the β lectum ring. The Subsequent generation of cephalosporins which could over come β lactamases are called extended spectrum of cephalosporins which include

oxyimino β lactum like Ceftazidime and cefotaxime. Resistance to the antibiotics are by synthesis of extended spectrum of β lactamases (ESBL) which are plasmid mediated, these enzymes different from their parent enzymes by only few amino acids portion but can hydrolyse extended section of cephalosporins.

Though these plasmid mediated β lactamases are observed in pseudomonas areuginosa isolates, a new class of chromosomally encode section of β lactamases also reported. These enzymes have moderate hydrolysis activity for oxacillin but high activity against extended spectrum cephalosporins cefotaxime and ceftazidime.

As antibiotic resistance continues to escalate and speed with a vengeance in our environment, our complacency, remisistant of behaviour in the peresistence were in handling our greatest resource the "miracle drugs" is no longer acceptable. Increasing resistance requires us to manage the infectious disease in a more ecologically conscious manner. It is necessary to promote the rational use of antibiotics.

So it is need of the hour to evaluate the multidrug resistant pseudomonas aeruginosa in chronic suppurative otitis media. Against this back ground we evaluate the multi drug resistant among pseudomonas aeruginosa isolates from chronic suppurative otitis media.

As Pseudomonas aeruginosa is the most prevalent gram negative organism in CSOM. Production of various enzymes by them may require alteration in the management profile of gram-negative chronic Suppurative otitis media infection.

Hence we believe that our study will definitely enlight and advance in the management of multidrug resistant pseudomonas infection in middle ear and work towards reducing the morbidity and mortality by which we can reduce the hearing loss in many patients residing in developing countries like India.

AIMS & OBJECTIVES OF THE PRESENT STUDY

a) Isolation of aerobic causative organisms from chronic suppurative otitis media.

b) Identification of pseudomonas aeruginosa from chronic suppurative otitis meida and their characterization.

c) Determination of minimum Inhibitory concentration of selected expanded 3rd and 4th generation, for pseudomonas aeruginosa isolation from CSOM.

d) Detection of group I inducible β – Lactamase production by the test isolates and their prevalence.

e) Detection of extended spectrum of β –Lactamases (ESBLs) producers among the pseudomonas aeruginosa isolates from CSOM and their prevalence.

f) To evaluate synergy effects of selected antibiotics.

g) Comparative study of Multidrug resistant between Pseudomonas aeruginosa and other isolates from CSOM.

h) To isolate plasmids from CSOM strains of Multidrug resistant pseudomonas aeruginosa.

Review of Literature

2.1 Chronic Suppurative otitis media

Ear is the most important sensory organ concerned with perception of sound. Infections of Ear are the leading causes of deafness in India.

Chronic suppurative otitis media is a persistent inflammation of middle ear of mastoid cavity. It is characterised by recurrent or persistent ear discharge over 2 – 6 weeks through a perforation tympanic membrane (Plate 2). Typical findings also include thickened granular middle ear mucosa, mucosal polyp, and cholesteotoma with in the middle ear (Scott and brown 5th edition, text book of otology). The incidence of chronic suppurative otitis media appears to depend socio economic factors such as poor living conditions, over crowding, poor hygiene and nutrition have been suggested as the basis for the wide spread prevalence of chronic suppurative otitis media in the developing countries like India. Chronic suppurative otitis media is traditionally classified into two main groups Tubo tympanic and attico antral diseases. Tubo tympanic disease was considered safe from complication while the attico antral disease was considered to be a dangerous and unsafe form of disease in which intracranial complications are possible.

The wide of microbes both aerobic and anaerobic present in chronic suppurative otitis media. The types of aerobic microbes isolated in chronic suppurative otitis media are pseudomonas, proteus, E. Coli, Klebsiella, staphylococcus aureus and anaerobes are bacteroid melanino genicus, B. Fragilis, pepto streptococcus and propionibacterium (Brobby Gwetal; 1987.

Brook I et al; 1996).

2.2 Pseudomonas aeruginosa

Pseudomonas aeruginosa are aerobic motile straight and slender gram-negative bacilli range from 1-5 mm in length and 0.5 to 1mm in width. They use variety of carbohydrate, alcohol and amino acid substrates as carbon and energy sources. Although they are able to survive and possibly grow at relatively low temperatures (as low as 4ºC) they are mesophilic (optimum temperatures for growth is between 30 to 37ºC) .

Pseudomonas aeruginosa can be grown in ordinary medium like nutrient agar. On nutrient agar the colonies are large, circular, flat with irregular spreading margins, The Colonies show metallic tinge and produce diffusible phenazin pigment when grown.

In Macconkey agar the organism grow as transparent, spreading non-lactose fermenting colonies with metallic tinge. In broth the organism produces thick surface pellicle while growing and pigment diffuses from surface of medium.

Biochemically pseudomonas aeruginosa behaves as a non-fermenter breaking down glucose and other sugars oxidatively, which can be detected in amino sugar media. Prompt oxidase positivity, citrate utilization, Pigment production, growth at 42ºC and non- fermenting reactions help in confirmation of this organism, they are widely present in nature. They have isolated from Soil, water, plants, hot tubs, whirl pools and hospital environments. Rarely they

are part of normal flora in healthy individuals. The transmission is known to occur through ingestion of contaminate food and water, exposure to contaminated mediated devices and solution, introduction by penetrating wounds and person to person transmission.

Pseudomonas aeruginosa infections in the ear are an important public health problem. The intrinsic virulence of an organism relates its ability to invade tissue, resist host defense mechanisms and produce tissue damage. Disruption of normal ear surface predisposes the ear to microbial adherence invasion and infectivity. Pseudomonas infection in chronic suppurative otitis media (Tubo tympanic type) is the commonest disease, causing deafness.

2.3. CEPHALOSPORINS

Some cephalosporium fungi yield antimicrobial substances called cephalosporins. These are (β-lactam compounds with a nucleus of 7-aminocephlosporanic acids, instead of the penicillins' 6-aminopenicillanic acid. Natural cephalosporins have low antimicroibial activity, but the attachment of various R side groups has resulted in the proliferation of an enormous array of drugs with varying pharmacologic properties and antimicrobial spectra and activity.

2.3.1 MECHANISM OF ACTION

The mechanism of action of cephalosporins is analogous to that of penicillins:

- Binding to specific PBPs that serve as drug receptors on bacteria

- Inhibiting cell wall synthesis by blocking trans peptidation of peptidoglycon
- Activating autolytic enzymes in the cell wall that can produce lesions resulting in bacterial death.

2.3.2 RESISTANT TO CEPHALOSPORINS

Resistant to cephalosporins can be attributed to:

- Poor permeation of bacteria by the drug
- Lack of PBP for a specific drug
- Degradation of drug by β-lactamases

2.3.3 COMMONLY USED CEPHALOSPORINS

For easy reference, cephalosporins have been arranged into three major groups or generations. Many cephalosporins are mainly excreted by the kidney and may accumulate and induce toxicity in renal insufficiency.

2.3.4 FIRST GENERATION CEPHALOSPORINS

First generation cephalosporins are very active against gram-positive cocci except enterococci and methicillin resistant Staphylococci and meditatively active against some gram-negative rods-primarily E. coli, Proteus, and Klebsiella. Anaerobic cocci are often sensitive.

(Example: Cefazolin, Cephalexin, Cephradine, Cefdraxil

2.3.5 SECOND GENERATION CEPHALOSPORINS

The second-generation cephalosporins are a heterogeneous group. All are active against organisms covered by first generation drugs but have extended coverage against gram negative rods including Klebsiella,

Enterobacter, and Proteus but not P. aerugirosa

(Example: Cefamandole, Cefoxitin, Cefuroxime)

2.3.6 THIRD GENERATION CEPHALOSPORINS

Third generation cephalosporins have little activity against gram-positive cocci. These drugs are very useful in the management of hospital acquired gram-negative bacterimia.

(Example: Cefotaxime, Ceftazidime, Ceftriaxone)

2.3.7 FOURTH GENERATION CEPHALOSPORINS

Some new cephalosporins classified as fourth generation drugs. The new agents have comparable or slightly enhanced activity against some enterobcateriaceae that are resistant to third generation cephalosporins. They are not active against P. aeruginosa that are resistant to third generation drugs.

(Example: Cefepime)

The significance of P. aeruginosa lies in its ability to develop resistance to various antibiotics currently used in clinical practice, in particular to extended spectrum β-lactamases. Their resistance is mainly due to synergy between multi-drug efflux systems, β-lactamases production, and low outer membrane permeability or through a combination of multiple unrelated resistance mechanism. Out of these resistance mechanisms the current research is on production of β-lactamases and extended spectrum of β-

lactamases. β-lactams account for approximately 50% of global antibiotic consumption and this heavy usage exerts considerable selective pressure for resistance arising via production of β-lactamases.

β-lactamases are the commonest cause of bacterial resistance to β-lactam antimicrobial agents. Many β-lactamases are known, and at present there are about 190 types. Most function by a serine ester

2.4 CLASSIFICATION OF β-LACTAMASES

β-lactamases have been classified by their hydrolytic spectrum, susceptibility to inhibitors, and whether they are encoded by the chromosome or by plasmids. Also whether cephaloridine is hydrolyzed more rapidly than benzylpenicillin, or vice versa, and on whether an enzyme is inactivated by cloxacillin, clavulanate, aztreonam, or p-chloromercuribenzoate. Though various classification were put forward a major reorganization and an updated classification of β-lactamases was proposed by Bush in 1995 (Bush et al, 1995). The revised Bush scheme classifies β-lactamases by their substrate preference among penicillin, oxacillin, carbenicillin, cephaloridine, expanded-spectrum cephalosporins, and imipenem and by their susceptibility to inhibition by clavulanate.

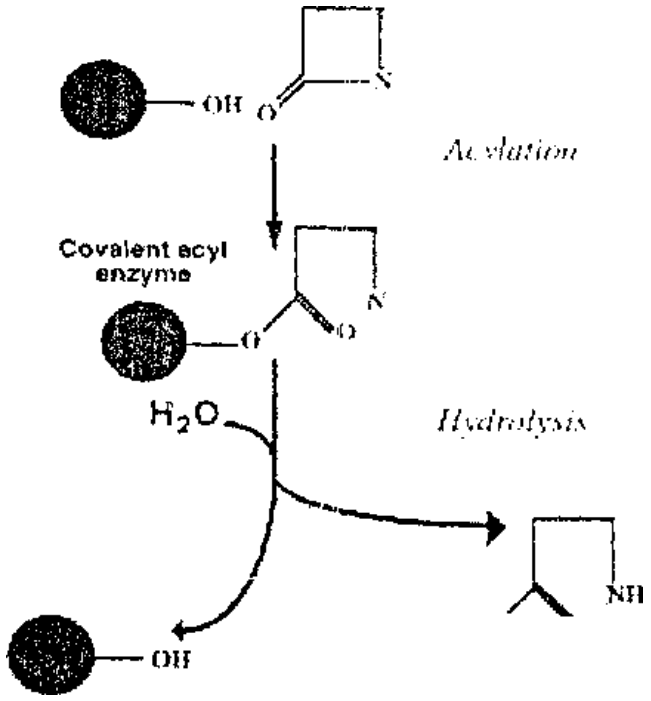

Fig. 1. Action of a serine (β-lactamase.

The enzyme first associates noncovalently with the antibiotic to yield the noncovalent Michaelis complex. The β-lactam ring is then attacked by the free hydroxyl on the side chain of a scrine: residue at the active Site of the enzyme, yielding a covalent acyl ester. Hydrolysis of the ester finally liberates active enzyme and the hydrolyzed, inactive drug. This mechanism is followed by β-lactamases of molecular classes A, C, and D, but class B enzymes utilize a zinc ion to attack the [β-lactam ring. (Livermore, 1995).

β-lactamases are classified by sequence, was first proposed by Ambler (Ambler, 1980). Such classification is stable, as it reflects fundamental relationships and cannot be distorted by mutations. Moreover, sequence-based classification has the beauty of simplicity, recognizing only four classes, designated A to D. Classes A, C, and D Comprise evolutionarily distinct groups

of serine enzymes, and class B contains the zinc types.

At present, classes recognized in Bush's phenotypic classification and those defined in the molecular scheme, except that Bush's group 2d includes a few class A enzymes from actinomycetes as well as all the class D types from gram negative rods.

2.5 FREQUENCY OF ENZYME PRODUCTION

Frequencies vary-hugely among countries, hospitals, unit types, and patient types. Resistance generally is most common where antibiotic usage is greatest (Sanders and Sanders, 1992) notably in intensive care units, hematology departments, and burns units, as well as in developing countries, where medical and surgical advances often outpace infection control. Conversely, resistance rates often are very low in the general wards of community hospitals of developed countries. Such differences are easy to rationalize in terms of the degree of selection pressure, as are high resistance rates in recalcitrant infections (e.g., P. aeruginosa in cystic fibrosis), in which bacteria are repeatedly exposed to antimicrobial agents. Rates of β-lactamase production also vary hugely in community-acquired pathogens from different geographic sources.

2.6 DEFINITION OF RESISTANCE

The effects of (β-lactamases on resistance sometimes are unequivocal. (β-lactamases reduce susceptibility without raising MICs above the breakpoint and, similarly, (β-lactamase inhibitors reduce MICs without restoring full susceptibility. Susceptibility and resistance can be defined on the basis of

either biological or pharmacological criteria (Livermore, 1995).

Biological breakpoints view an organism as resistant if the observed MIC or inhibition zone falls outside the normal distribution of MICs or zones for isolates without specific resistance mechanisms; (O'Brien, 1987; Williams, 1990) pharmacological breakpoints, favored by the National Committee for Clinical Laboratory Standards (Villanova, 2000) and the British Society for Antimicrobial Chemotherapy (Jennifer, 2001), define resistance relative to the drug concentration achievable in vivo.

Biological analysis automatically gives significance to small reductions in susceptibility and allows emerging low-level resistance to be detected and monitored, whereas pharmacological breakpoints may demand tiny or huge MIC changes before an organism is deemed to be resistant. For example, the NCCLS (pharmacological) breakpoint for cefoxitin of 16 mg/ml is only double the modal MIC for typical E. coli or Bacteroides isolates, whereas the breakpoint for cefotaxime of 8 mg/ml exceeds the MICs for typical enterobacteria by 50- to 100-fold. Examples can be found to defend either basis of choosing breakpoints, and it is worth citing two contrasting examples. First, many extended-spectrum TEM p-lactamases raise the MICs of cefotaxime and other extended-spectrum cephalosporins for enterobacteria to only 1 to 4 mg/ml, compared with 0.03 mg/ml for isolates without these enzymes (Jorgensen et al, 1990) Although the MICs for the producers are below the NCCLS breakpoint, the organisms commonly prove resistant in vivo (Rice et al, 1991). Such a situation is powerful ammunition to proponents of biological breakpoints, who can point' out that MICs for the enzyme producers

were 32- to 128-fold above the normal level. On the other hand, N. gonorrhoeae isolates which, through impermeability and target modification, are biologically resistant to penicillin (MICs of 0.25 to 1 mg/ml compared with 0.008 mg/ml for fully susceptible isolates) remain susceptible to high-dose penicillin in vivo. Here, the pharmacological breakpoint of 2 mg/ml is defensible, particularly since the β-lactamase-producing strains, which are unresponsive in vivo, tend to be more highly resistant, with MICs being around 16 ug/ml.

This review will indicate several instances in which pharmacological breakpoints give a falsely optimistic picture of susceptibility for β-lactamase producers and for which biological analysis is more appropriate, on the basis of clinical experience. Biological rather than pharmacological breakpoints must also be considered by anyone attempting to use antibiogram data to predict the resistance mechanisms present in clinical isolates. It should, however, be appreciated that biological breakpoints are easy to define when a susceptibility distribution is bimodal, with the MICs or zones for isolates with a β-lactamase (or other resistance mechanism) distinct from those for isolates without the enzyme, but are harder to set when the possessors of the mechanism merely form the tail of a skewed normal distribution (Williams, 1990). β-Lactamase-inhibitor combinations present a particular problem in this regard, as the inhibitors reduce the MIC of their partner penicillins for β-lactamase producers but generally fail to render the bacteria as susceptible as those without the enzyme (Livermore, 1993; sanders et al, 1988).

Some β-lactamase hyperproducers are resistant to inhibitor combinations, but the zonea or MICs for these organisms do not form a distinct distribution from those for organisms that have slightly less enzyme and that, consequently, are susceptible. Pharmacological analysis allows a breakpoint to be drawn in these circumstances, although its positioning may be highly arguable, as has been the case with ticarcillin-clavulanate (Sanders etal, 1988).

2.7 CHROMOSOMAL β-LACTAMASES OF PSEUDOMONAS AERUGINOSA

P. aeruginosa has an inducible AmpC enzyme, similar to that of Enterobacter spp. As with Enterobacter spp., ampicillin and narrow-spectrum cephalosporins are labile to hydrolysis and induce the enzyme strongly, destroying their own activity, whereas ureidopenicillins and extended-spectrum cephalosporins are labile but induce weakly and so are active against inducible strains but not against derepressed mutants (Livermore and Yang, 1987). Carbapenems are strong inducers that are marginally labile (imipenem) (Livermore and Yang, 1987) or are effectively stable (meropenem) (Livermore and Yang, 1989) and so remain active irrespective of the mode of (β-lactamase lactamase expression. Differences from the Enterobacter system are (i) that carbenicillin is less affected by derepression in P. aeruginosa, with its MICs increasing from 32 to 128 mg/ml for inducible isolates to 64 to 256 mg/ml for derepressed ones, as against from 1 to 2 mg/ml to 128 to 256 mg/ml, respectively, for (β-lactamase-inducible and derepressed Enterobacter spp. (Livermore and Yang, 1989; yang et al, 1988); (ii) that the P. aeruginosa

enzyme, whether inducible or derepressed, gives slight protection against imipenem, with MICs for producers being around 1 to 2 mg/ml compared with 0.12 to 0.5 mg/ml for β-lactamase-deficient laboratory mutants (Livermore, 1992); and (iil) that derepression in P. aeruginosa is often only partial, such that the uninduced enzyme level is higher than is normal for the species but substantial inducibility is retained (Sanders and Sanders, 1992; Williams et al, 1984), whereas derepression in Enterobacter spp.

Is almost always total, with the enzyme being manufactured constitutively. P. aeruginosa strains segregate partially derepressed mutants at rates of ca. 1027, but totally derepressed mutants occur at frequencies below 1029, whereas Enterobacter spp. segregate totally derepressed mutants at frequencies of 1025 to 1027. The resistance of partially derepressed isolates mirrors the amount of enzyme produced without induction; even a small degree of derepression compromises ureidopenicillins, whereas only total derepression noticeably compromises cefepime and carbenicillm (Williams et al, 1984).

Selection of totally or partially derepressed mutants can occur during antipseudomonal therapy with labile weak inducers. Ureidopenicillins and piperacillin, as well as extended spectrum cephalosporins, have been widely reported to select for these mutants in P. aeruginosa infections (Livermore, 1987), whereas selection of derepressed enterobacteria is predominantly by cephalosporins. Overall, however, selection of derepressed mutants is rarer with P. aeruginosa than with Enterobacter spp. and C. freundii, except under the specialized conditions of the lungs of patients with cystic fibrosis. Evolution during therapy from inducible through partially derepressed to totally

derepressed has been reported (Shannon et al, 1982), but it is unclear whether this is the invariable pattern or whether total derepression can also arise directly from inducibility.

2.8 PLASMID MEDIATED AND OTHER SECONDARY β-LACTAMASES OF NON FASTIDIOUS GRAM-NEGATIVE BACTERIA

2.8.1 DISTRIBUTION AND DIVERSITY:

Over 75 different plasmid-mediated β-lactamases have been recorded in gram-negative bacilli, (Bush et al., 1995) and numerous surveys of their frequency have been undertaken. The commonest of these enzymes in enterobacteria is TEM-1, which is responsible for most of the ampicillin resistance now seen in about 50% of E. coli isolates (Sanders and Sanders, 1992). TEM-2, SHV-1, and OXA-1 p-lactamases also are widespread in enterobacteria, although they are much rarer than TEM-1, and numerous other types have been seen in occasional isolates.

Secondary β-lactamases in P. aeruginosa have been reported widely but are much rarer than in enterobacteria. Thus, multicenter surveys in the United Kingdom found secondary β-lactamases in only 2.5% of 1,866 P. aeruginosa isolates collected in 1982 (Williams et al, 1984), incidence rates of 13 and 7% have been reported from France (Thabaut et al, 1985) and Spain (Tirado, 1986), respectively, but these last studies are over 10 years old. Aside from their scarcity, the other characteristic of secondary β-lactamases in P. aeruginosa is their diversity. PSE-1 and PSE-4 enzymes predominate in P.

aeruginosa (Thabaut et al, 1985; Tirado, 1986; Williams et al, 1984), largely because of the clonal selection of producers rather than because of plasmid spread (Livermore et al, 1985; Pitt et al, 1990). In addition, however, numerous OXA types have been recorded in P. aeruginosa, as have various more obscure types such as NPS-1 and LCR-1. TEM and SHV types do occur but are rare, in contrast to their predominance in enterobacteria.

The classical TEM-1, TEM-2, SHV-1, OXA-1, PSE-1, PSE-4 enzymes have minimal activity against newer cephalosporins, other than cefamandole and cefoperazone (O'Callaghan, 1979). In the past 10 years, however, there has been increasing emergence of "extended-spectrum" β-lactamases (ESBLs), which attacks many newer cephems and monobactams as well as narrow-spectrum cephalosporins and anti-gram-negative-bacterium penicillins (Jacoby and Medeiros, 1991 and Philippon et al., 1989). Most ESBLs are mutants of TEM-1, TEM-2, and SHV-1, with 1- to 4-amino-acid sequence substitutions. These changes, amounting to less than 2% of the protein sequence, sufficiently remodel the enzyme active site to allow attack on most or all aminothiazolyl cephalosporins.

Over 25 different TEM and SHV variants have been claimed and are numbered TEM-3 to TEM-27 and SHV-2 to SHV-7. They are commonest in klebsiellae but also occur in other enterobacteria. The first major outbreak due to producers, specifically isolates with TEM-3 β-lactamase, occurred around Clermont-Ferrand in 1985 to 1987 (Petit et al., 1990) and was soon followed by outbreaks elsewhere in France (Jacoby and Medeiros, 1991).

TEM-3 seems commonest in France (Petit et al, 1990; Philippon, 1989), whereas TEM-10, TEM-12, and TEM-26 predominate in the United States (Bradford et al, 1994; Naumouski et al, 1992; Rice et al, 1993). SHV variants are also important worldwide. SHV-2 and SHV-5 enzymes have each been recorded in at least five countries (Jacoby and Medeiros, 1991), with the latter type widespread in Greece (Gianneli et al, 1994). A single serotype K25 K. pneumoniae strain with SHV-4 β-lactamase has, been transferred among many hospitals in France (Alert et al, 1994).

Other ESBLs besides TEM and SHV derivatives are emerging, but these presently are very rare. They include representatives of all four molecular classes. Yet another recent development is the emergence of mutants of TEM and SHV (3-lactamases that lack ESBL activity but are resistant to inhibition by clavulanate and penicilianic acid sulfones.

It should be emphasized that the dissemination of plasmid mediated (3-lactamases in gram-negative bacteria is very recent. TEM-1 enzyme was first reported from a single E. coli isolate in 1965, and the earliest known ESBLs date from 1982 to 1983 (Kliebe et al, 1985). There can be few examples of faster evolution than the spread of these enzymes.

2.9 EXTENDED-SPECTRUM TEM AND SHV β-LACTAMASES

The most notable feature of these enzymes, distinguishing them from their TEM-1, TEM-2, and SHV parent types, is their ability to attack extended-spectrum cephalosporins and monobactams, as well as narrow-spectrum cephalosporins and anti-gram-negative- bacterium

penicillins (Jacoby and Medeiros, 1991; Philippon et al., 1989).

Carbapenems and cephamycins are stable, as is temocillin. Ceftibuten is also stable to most types, except to a few SHV derivatives (Jacoby and Carreras, 1990).

All the TEM- and SHV-derived ESBLs confer antibiograms that reflect this general pattern of activity, but individual enzymes vary in the levels of resistance they cause to different compounds. Some types, including TEM-3 and SHV-2, give clear resistance (MIC, .16 mg/ml) to all extended-spectrum cephalosporins and to aztreonam (Jacoby and Carreras, 1990); others, including the TEM-10 and TEM-26 types that currently predom- inate in the United States, give clear resistance to ceftazidime (MIC, >128 mg/ml) but raise the MICs of cefotaxime, ceftriaxone, cefpirome, and ceftizoxime to only around 0.5 to 4 mg/ml (Jacoby and Carreras, 1990; Katsanis et al, -1994; Liu et al., 1992; Rice et al, 1993; Rice et al, 1990; Rice et al, 1991). TEM-12, which is the evolutionary ancestor of TEM-10 and TEM-26, is even feebler, generally raising the MICs of ceftazidime for E. coli and klebsiella isolates to only 4 to 8 mg/ml and leaving- those of cefotaxime and .ceftriaxone at around 0.06 to 0.25 mg/ml (Jacoby and Medeiros, 1991; Katsanis et al, 1994; Rice et al., 1993; Rice et al, 1990), although giving greater resistance, especially to ceftazidime, in a porin-deficient strain (Weber et al, 1990). Producers of TEM-10, TEM-12, and TEM-26 enzyme types thus have biological resistance to the many newer cephalosporins, being up to 100-fold less susceptible than are strains without enzyme, but remain apparently susceptible at the breakpoints advocated by the NCCLS (8 to 16 mg/ml) (Katsanis et al, 1994; Rice et al, 1991). This can

lead to serious interpretive problems.

Various more or less complicated tests have been advocated, but the most practicable is simply to screen with ceftazidime, since virtually all ESBLs give clear resistance to this compound (Katsanis et al, 1994). When isolates have reduced susceptibility to ceftazidime, double-disc tests can be used to screen for synergy between this compound and clavulanate, which is most conveniently available in amoxicillinclavulanate (20 plus 10, mg) discs. When the ceftazidime zone is expanded by the clavulanate, production of an ESBL is inferred. Such testing would be facilitated if discs combining ceftazidime and clavulanate were available, as one could simply compare the zones with and without inhibitor. E-test ellipseometers and Vitek cards with this combination are under development, and early results indicate that they allow accurate detection of ESBL producers.

Carbapenems are stable to ESBLs, and imipenem has been used successfully in vivo against many enzyme producers. Inhibitor combinations and cephamycins may also overcome these enzymes, but their efficacy is more controversial. Despite sensitivity of the enzymes to inhibition, MICs of clavulanate combinations and ampicillm-sulbactam often are high for ESBL producers (Jacoby and Carreras, 1990; Jacoby and Medeiros, 1991), presumably because high levels of enzyme are present. Piperacillin-tazobactam seems a better prospect than other inhibitor combinations, at least against isolates with TEM derivatives, most of which are susceptible to the combination at 16 plus 4 mg/ml (Jacoby and Carreras, 1990). Moreover, piperacillin- tazobactam was effective against a K. pneumoniae strain with

TEM-3 enzyme in a rabbit endocarditis model, whereas unprotected piperacillin was inadequate (Leleu et al, 1994). Whether or not piperacillin-tazobactam is a viable option against producers of SHV-derived ESBLs is less certain: two groups (Bauernfiend, 1990; Jacoby and Carreras, 1990) have reported that E. coli and K. pneumoniae strains and transconjugants with SHV-2, SHV-3, SHV-4, and SHV-5 enzymes typically were resistant to piperacillin- tazobactam, with MICs ranging upwards from 64 plus 4 mg/ml.

Cephamycins are stable to ESBLs, and the continued activity of these compounds, but not of other cephalosporins, facilitates laboratory recognition of ESBL producers (Jacoby and Carreras, 1990). Nevertheless, failures were reported when cefoxitin was used against ESBL producers and were associated with selection of porin-deficient mutants (Pangon et al, 1989). It is unclear whether this risk would arise with cefotetan or moxalactam, which share the stability of cefoxitin to ESBLs but have 10- to 100-fold-greater antienterobacterial activity. Perhaps it is significant that there have been few reports of ESBL producers in Japan, where moxalactam has long been a favored drug, and many reports from France and the United States, where cefotaxime, ceftriaxone, and ceftazidime have been preferred.

2.10 INHIBITOR-RESISTANT TEM MUTANTS

In addition to their ESBL derivatives, TEM--1 and TEM-2 [β-lactamases segregate mutants that have reduced affinity for clavulanate and the sulfones. Ten variants have been described (Blasquez et al, 1993) and, to add confusion, have been numbered variously as IRT (inhibitor-resistant-TEM) types 1, 2, 3, etc.; as TRI (TEM, resistant to inhibitors) types 1, 2, 3, etc.; and as TEM types 30, 31, 32, etc.! Producer strains are resistant to all β-

lactamase- inhibitor combinations but are less resistant than isolates with classical TEM-1 enzyme to narrow-spectrum cephalosporins and remain fully susceptible to extended-spectrum cephalosporins.

At present, resistance to inhibitor combinations is more often caused by high-level production of TEM-1 enzymes (Seetul singh et al, 1991) than by these mutants, but this situation may change as more potent inhibitor combinations, such as piperacillin-tazobactam and cefoperazone-sulbactam, are increasingly used.

2.11 EXTENDED-SPECTRUM SECONDARY β-LACTAMASES NOT RELATED TO TEM AND SHV.

Among extended-spectrum class A (3-lactamases that are not TEM and SHV derivatives are PER-1 (Danel et al, 1995; Nordmann et al, 1993), its close relative CTI-1 and MEN-1 (Bernard et al, 1992). Each gives resistance to all cephalosporins, aztreonam, and penicillins but spares carbapenems and cephamycins. CTI-1 and MEN-1 have been detected only in single isolates, but PER-1 appears well established in Turkey, having been found in 14 P. aeruginosa isolates collected over an 18-month period at an Ankara teaching hospital and in salmonellae from Istanbul (Danel et al, 1995). It has not been seen elsewhere, except in a P. aeruginosa isolate from a Turkish patient who was transferred to Paris (Nordmann et al, 1993). Its gene has been recorded on at least three different plasmids (Danel et al, 1995), suggesting transposition. Isolates with PER-1 enzyme have a characteristic antibiogram, being highly resistant to ceftazidime (MIC, .256 nag/ml)-but very susceptible to

ceftazidime- clavulanate (MIC, 1 to 4 mg/ml) and having only slightly reduced susceptibility to piperacillin (MIC, 8 to 16 mg/ml) as compared with typical P. aeruginosa isolates. Other exotic class A enzymes include three related carbapenemases, carbapenemases, Sme-1, Imi-1, and NMC-A, all of which are encoded by nontransferable chromosomal inserts. Sme-1 was from two Serratia marcescens isolates obtained in London in 1982 (Naas et al, 1994); NMC-A was from a single B. cloacae isolate obtained in Paris in 1990 (Nordmann et al, 1993). Each attacks and gives resistance to penicillins, aztreonam, and carbapenems, with imipenem being compromised more than meropenem.

Aminothiazolyl cephalosporins are largely spared, and the enzymes are subject to inhibition by clavulanate.

Plasmid-encoded class C enzymes have been reported from klebsiellae and E. coli isolates and represent cases where the chromosomal β-lactarnase genes of Enterobacter spp. and C. freundii have escaped on extrachromosomal elements. The first claim of a plasmid-mediated AmpC enzyme dates from 1976 (Bobrowski et al., 1976), but the strain and its putative transconjugant were subsequently lost. Since 1989, however, several plasmid-encoded AmpC enzymes have been documented, including MIR-1 from U.S. isolates (Papanicolaou et al, 1990), CMY-2 from South Korea (Bauernfiend, 1989) BIL-1 from Pakistan (Payne et al, 1992), FOX-1 from Argentina (Leiza et al, 1994), LAT-1 from Greece (Tzouvelakis et al, 1993), FEC-1 (Matsumoto et al, 1988) and MOX-1 (Horii et al, 1993) from Japan, and CMY-3 from the United Kingdom (Jenks et al, 1995). Producers have

antibiograms resembling those of derepressed Enterobacter strains, with resistance to all β-lactams except carbapenems, temocillin, and mecillinam. Resistance usually is not reversed by clavulanate and sulbactam, although FEC-1 and MOX-1 provide exceptions to this generalization. The lack of susceptibility to cephamycins and inhibitor combinations distinguishes such producers from isolates with TEM- and SHV derived ESBLs. The fact that producers of these enzymes have been reported from many different countries in a very brief period suggests that a real problem may be developing. Finally, extended-spectrum activity has been found in mutants of a class D enzyme, OXA-10 (PSE-2). One such mutant, OXA-11, has been reported by Hall et al (Hall et al., 1993). All are from P. aeruginosa isolates obtained at one hospital -in Ankara, Turkey. OXA-10 itself has a broader spectrum than other OXA types, giving high-level resistance to cefoperazone and, if hyperproduced, causing small reductions in susceptibility to aztreonam, cefotaxime, and ceftriaxone. Its extended-spectrum mutants give greater resistance to cefotaxime, ceftriaxone, and aztreonam and cause high-level resistance to ceftazidime (MIC, 256 to 512 mg/ml), which is spared by OXA-10 itself. OXA-10 and its derivatives are poorly inhibited by clavulanate or sulfones. Carbapenems are stable to their activity.

2.12 TESTS FOR (β-LACTAMASE PRODUCTION

Many tests for β-lactamase production, entailing either chromogenic reactions or detection of the destruction of antibiotic activity have been proposed. The chromogenic methods are faster and more convenient, and they divide into those in which the hydrolysis of the β-lactam itself engenders a

color change and those in which this change depends on a linked reaction.

In the former group are nitrocefin (O'Callaghan et al, 1972), which changes from yellow to pink/red on hydrolysis, and 7-(thienyl-2-acetamido)-3-[2-(4-N, N-dimethylaminophenylazo) pyridinium methyl]-3-cephem-4-carboxylic acid (PADAC), which changes from violet to yellow. Of these two, nitrocefin is the more readily available (albeit expensively) and can be used as a solution or as discs upon which test cultures are smeared. It is highly sensitive to most β-lactamases, although false-negative results are a risk for /-/. influenzae isolates with ROB-1 β-lactamase and for staphylococci, in which uninduced penicillinase levels are often inadequate to give a color reaction. These problems are minor, since ROB-1 enzyme is rare and β-lactamase tests are rarely performed on staphylococci. Linked detection systems include the iodometric and acidimetric methods. The former depends on the fact that the hydrolysis products of β-lactams reduce iodine to iodide; consequently, decolorization of starch-iodine complex occurs if an isolate is a β-lactamase producer but not if the enzyme is absent. Acidimetric tests depend on the fact that opening the β-lactam ring generates a free car'boxyl and that this acidity can turn bromocresol purple from violet to yellow in an unbuffered system. Acidimetric and iodometric tests can be performed with bacterial suspensions or on paper strips impregnated with the appropriate reagents. These methods are cheaper than nitrocefin and, given care, almost as sensitive, but they are more prone to false-positive results. With iodometric tests, such errors probably reflect nonspecific reaction of iodine with bacterial proteins; for

acidimetric tests, false-positive results arise if the inoculum or the distilled water used to moisten the test strip is slightly acidic. More generally, these problems underscore the importance of performing parallel controls with known enzyme producers and nonproducers.

Such β-lactamase detection tests do not provide any information about what type of β-lactamase is present. Preliminary typing can, however, be achieved by running parallel tests in the presence and absence of 0.1 mM clavulanate and 0.1 mM cloxacillin (Williams et al, 1984); most class A enzymes are inhibited by clavulanate but not cloxacillin, whereas class C enzymes give the opposite pattern; class B and most class D enzymes are inhibited by neither compound. These method's are, however, most useful for preliminary β-lactamase, typing in surveys rather than routine use. Their main limitation is that increasing numbers of isolates have multiple [3-lactamases and that these, obviously, can distort the inhibition 'patterns observed. Aside from chromogenic methods, β-lactamase production may be detected by various biological methods, which depend on the β-lactamase produced by one organism allowing an indicator strain to grow. Examples include "clover leaf plates" and Masuda double-disc tests (Masuda et al, 1976). These tests are extremely sensitive but are slow and tedious. More useful biological methods are the double-disc tests used to examine for synergy between clavulanate and ceftazidime and, thereby, to detect ESBLs.

Isolation, Screening and Characterisation of pseudomonas aeruginosa from CSOM specimens

3.1 SPECIMENS

All the Ear Swab specimens obtained from CSOM patient were processed in the Department of microbiology Government medical college hospital Coimbatore, South India from October 2005 to September 2006 were considered for the study, A total of 43 Pseudomonas aeruginosa isolates from chronic suppurative otitis media patients were cultured and they were stored as suspensions in a 10% (wt/vol) skim milk solution (STGG medium) containing 10% (Vol / Vol) glycerol at 80°C until additional tests were performed.

COMPOSITION OF STGG MEDIUM

Skim milk powder	-	2 gm
Triptycase soy broth	-	3 gm
Glucose	-	0.5 gm
Glycerol	-	10ml
Distilled water	-	100ml.

3.2 SPECIMEN COLLECTION

The Bacteriological cultures were obtained from CSOM patients as per standard procedure. All the exudates and necrotic materials were removed from the external auditory canal using sterile cotton moist swab. Single use minitip culture swabs were used to harvest middle ear micro flora under vision

and were transported two Ear swabs to the laboratory without delay. The status of the external auditory canal and tympanic membrane was inspected to determine the disease entities (ie otitis media). Two Swabs were collected from each patient and one was used to spread on clean glass slide and stained with Gram's Stain for immediate microscopic examination.

The other swab was inoculated immediately to blood Agar, chocolate Agar, Macconkey Agar, Potato dextrose Agar, Cetrimide Agar and Brain heart infusion broth for bacteria isolation. "C" shaped streaks in a row were made with spatula while inoculating solid media in order to obtain inoculums in decreasing gradation.

3.3 INCLUSION CRITERIA

1) Recurrent or persistent ear discharge over 2-6 wks through a perforation of tympanic membrane who had not received Topical or systemic antibiotic therapy for the previous five days

2) Patients age ranged from 1 month to 80 years

Their pathogenic potential was confirmed by correlating with clinical findings and repeat isolations.

3.4 EXCLUSION CRITERIA

1. Acute otitis media
2. Chronic otitis media with effusion
3. Cases having purely sanguineous or CSF otorrhoea.
4. Antibiotic therapy (Topical or systemic) with in previous five days.

3.5 SPECIMEN PROCESSING

The specimens were processed as per standard protocol (Forbes et al, 1998; Sharma, 1988) for isolation of bacterial and fungal organisms.

3.6.1 GRAMS SMEAR FROM THE SPECIMENS AND CULTURE

SMEAR PREPARATION

1. The clinical material ear swab was spread thinly and uniformly over a clean glass slide with the help of sterile inoculation loop.
2. Smears from liquid cultures were made by spreading a loopful of the culture on a slide.
3. Smears from culture on solid media were prepared by emulsifying the colonies in a drop of saline placed on the slide and spread over thinly.
4. Smear was allowed to dry in air and then heat fixed by gently passing over a flame once or twice.

3.6.2 GRAM STAINING (Plate 3)

1. The heat fixed smear was flooded with crystal violet and kept for 60 seconds.
2. The stain was poured off. Then the smear was washed with sterile distilled water and covered with Gram's iodine for 60 seconds.
3. Again the smear was washed with distilled water and decolorized with Acetone-alcohol mixture for not more than 10 seconds.
4. The smear was washed with distilled water and counter stained with carbol fuschin for 30 seconds.
5. Then the smear was again washed with distilled water, air-dried and

observation was made under oil immersion (100x).

REAGENTS:

PRIMARY STAIN:

Crystal violet - 10 gm

Absolute alcohol - 100 ml

Distilled water - 1 litre

MORDANT:

Iodine - 10 gm

Potassium iodide - 20 gm

Distilled water - 1 litre

DECOLOURIZER: ACETONE-ALCOHOL MIXTURE

Acetone - 50 ml

95% ethanol - 50 ml

COUNTER STAIN:

Carbol fuschin strong - 100 ml

Distilled water - 1 litre

3.7 MEDIA INOCULATION AND INCUBATION

The following media were included for isolation of the organisms; Blood agar, Chocolate agar, MacConkey agar, nutrient agar, cetrimide agar, brain

heart infusion broth and Potato Dextrose Agar.

All the plates and liquid media were incubated overnight at 37' C and plates were observed bacterial growth (Plate 5, 6). Plates showing confluent growth in more than one media simultaneously were considered for the study. Psudomonas aeruginosa colonies produced a yellow green pigment in cetrimide agar (Plate 7). The inoculated Potato Dextrose Agar was incubated at 27' C for fungal isolation.

MEDIUM COMPOSITION

TRYPTOSE BLOOD AGAR

Tryptose	-	10 g
Beef extract	-	3 g
Yeast extract	-	3 g
Sodium chloride .	-	5 g
Agar	-	15 g
Distilled water	-	1 litre
pH	-	7.1

The medium was sterilized and allowed to cool to 45ºC and aspetically added 5% defibrinated sheep blood. Mixed thoroughly and poured into sterile Petri plates and stored in refrigerator.

CHOCOLATE AGAR

Tryptose blood agar medium was prepared and cooled to 45°C, to which 5% blood was added, mixed thoroughly and was heated in a water bath at 80°C until the medium got a 'chocolate' colour. The medium was then poured into sterile petri plates and stored in refrigerator.

MacConkey AGAR

Peptic digest of animal tissue	- 17 g
Protease peptone	- 3 g
Lactose	- 10 g
Bile salts	- 1.5 g
Sodium chloride	- 5 g
Neutral red	- 0.03 g
Agar	- 15 g
Distilled water	- 1 litre
pH	- 7.1

POTATO DEXTROSE AGAR

Potatoes infusion from	- 300 g
Dextrose	- 5.5 g
Agar	- 15 g
pH	- 5.6

NUTRIENT AGAR

Peptic digest of animal tissue	- 5 gm
Beef extract	- 1.5 gm
Yeast extract	- 1.5 gm

Sodium chloride	-	5 gm
Agar	-	15 gm
Distilled water	-	1 litre
pH	-	7.4

3.8 BIOCHEMICAL CONFIRMATION

The confirmation of P. aeruginosa was based upon production of characteristic pigments (blue and green) (Plate 4). Additional biochemical tests used to identify P. aeruginosa included: Catalase, oxidase, Motility, oxidation of glucose on OF-medium, and growth in cetrimide agar (Forbes et al, 1998; Collee, 1996).

MOTILITY TEST: HANGING DROP PREPARATION

1. The motility of the organism was studied using hanging drop preparation.

2. A concave slide was taken and soft petroleum jelly was applied to the surface of the slide encircling the convexity.

3. A Cover slip was taken and a drop of liquid culture was placed on the center of cover slip.

4. The cover slip was inverted over the cover slip in such a way that the cover slip got adhered to the slide.

5. Then the slide was placed on the mechanical stage of microscope with the organisms were tested for motility first under low-power objective and then with high power objective.

CATALASE TEST

1. A small amount of over night culture was transferred on to the clean glass slide with a sterile wooden stick.

2. Immediately a drop of 3% hydrogen peroxide was placed onto a portion of a colony on the slide.

3. Evolution of bubbles from the colony was considered as a positive test.

REAGENT: 3% HYDROGEN PEROXIDE

30% Hydrogen peroxide solution was diluted to have a concentration of 3%.

OXIDASE TEST (Plate 8)

A small portion of colony was removed with wooden stick and rubbed with the growth on filter paper containing oxidase reagent.

REAGENT: 1% TETRAMETHYL-P-PHENYLENE-DIAMINE

DIHYDROCHLORIDE

Whatman No. 1 filter papers were soaked in a freshly prepared 1% tetramethyl-p-phenylene-diamine dihydrochloride. After 30 seconds the strips were freeze dried and stored in a dark bottle tightly.

OXIDATION OF CARBOHYDRATE TEST

MEDIUM

Peptone	-	2.0 g
Sodium chloride	-	5.0 g
Dipotssium hydrogen phosphate	-	0.3 g
Bromothymol blue (1% aqueous solution)	-	3 ml
Agar	-	3 g
Distilled water	-	1 litre

The pH was adjusted to 7.1 before adding bromothymol blue and the medium was sterilized in flask at 121°C for 15 minutes. The carbohydrate (glucose) to be added was sterilized separately and added to give a final concentration of 1%. The medium was then tubed to a depth of about 4cm. Medium was inoculated by stabbing and incubated at 37°C. Changing the colour of the medium begins at the surface and gradually extends downwards was considered as positive oxidation reaction.

CETRIMIDE AGAR

Pancreatic digest of gelatin	-	20 g
Magnesium chloride	-	1.4 g
Potassium sulphate	-	10 g
Cetrimide	-	0.3 g
Agar	-	15 g
Distilled water	-	1 litre
pH	-	7.2

3.9 MINIMUM INHIBITORY CONCENTRATION (Plate 10) DETERMINATIONS

3.9.1 ANTIMICROBIAL AGENTS

Antibiotics gradient strips (E: test) agar inoculation technique was used to determine the MIC. Himedia Hicomb MIC test strips were used in this study. Which is based on an innovative antimicrobial gradient provides precise and accurate assessment of antimicrobial activity against both fastidious and non-fastidious microorganisms.

3.9.2 PRICIPLE AND INTERPRETATION

The antibiotic gradient is created on the strip by applying different concentrations of antibiotics in repeated ways of an increasing number of small dots. When applied to the agar surface, the antibiotic diffuses in to the surrounding medium in high to low amounts from one end of the strips to the other. The gradient remains stable after diffusion and the Zone of inhibition created takes the form of ellipse. The MIC is read only on the side of the comb at the point where the zone edge meets the strip edge.

3.9.3 TECHNIQUE OF HICOMB MIC TEST

Muller – Hinton agar was prepared and sterilised at 121°cfor 15 minutes. The pH of the each batch of Muller – Hinton agar was checked when the medium was prepared. Then medium allowed to cool to 45 to 50°c in a water bath, The plates were poured to a depth of 4mm as quickly as possible to prevent cooling and partial solidification in the Container. The agar was

allowed to solidify at room temperature. After the plates are solidified some representative plates were incubated at 37ºC for 48 hours to check sterility. The remaining agar plates were refrigerated until needed. The plated were used after sterility check. The refrigerated plates were allowed to cool to get the room temperature before the use.

3.9.3 CONTROL PLATES.

Drug free plates prepared from the base medium were used as growth controls.

3.10. ANTI MICROBIAL CONCENTRATION AND INOCULAM PREPARATION

MIC for cefotaxime, Ceftazidime was determined by an antimicrobial gradient strips (E. test) agar inoculation technique on Muller – Hinton Agar. The gradient Concentration of Cefotaxime and ceftazidime is 240 120,60, 30, 15, 10, 7.5, 5, 3, 1, .1, .01, 0.001µg/ml,. The Test inoculam was Prepared with an over night growth of each isolate, which was adjusted to a turbidity equivalent to 0.5 McFarland standard, The test organism was inoculated in Muller – Hinton Agar plate. The inoculam was allowed to dry for 5 minutes with lid in place. The Hicomb MIC strip was applied on agar surface with the MIC scale facing down wards. Then plates were incubated at 37ºC and examined after 24 hours.

MULLER – HINTON AGAR

Beef infusion	–	300 ml
Casein Hydrolysate	-	17.5 gm

Agar	-	15 gm
Distilled water	-	1 Litre
Final pH	-	7.4

0.5 McFarland Standard solution

Barium Chloride 1 % Solution	-	0.05
Sulfuric Acid 1% Solution	-	9.95 Ml

3.11 INTERPRETATION OF RESULTS.

The zone of inhibition created takes the form of ellipse. The MIC was read only on the side of the Comb at the point where the zone edge meets strip edge.

Pseudomonas aeruginosa ATCC 27853 was used as a reference strain in every batch of MIC tests.

3.12 DETECTION OF GROUP I INDUCIBLE β -LACTAMASES

(Plate 11)

β -lactamases was investigated by disc approximation method ((Miles, 1996; Qin, et al, 2004; Collee, 1996). Cefotaxime (30 ug) disc was placed at distances 25 and 20 mm,respectively from a central (center to center) from the cephoxitin (30 pg) disc on Muller-Hinton agar plate inoculated with the test organism, After overnight incubation, distinct flattening of the inhibitory zone around the ceftazidime disc on the side nearest to the cephoxitin disc was regarded as the presence of inducible β-lactamase.

3.13 DETECTION OF EXTENDED SPECTRUM p-LACTAMASE (ESBL) ACTIVITY (Plate 12)

Strains were screened for-the presence of ESBLs by the double-disc synergy method (Miles, 1996). Three ceftazidime (30 ug) discs were placed at distances 20, 15, and 10 mm, respectively, from a central amoxicillin-clavulanic acid disc. The test result was considered positive when an enhancement of the inhibition. zone around at least one of the ceftazidime disc toward the clavulanic-acid disc was observed as described by Bert (Bert et al, 2003).

3.14 INVESTIGATION OF SYNERGY EFFECTS OF ANTIBIOTIC COMBINATIONS (Plate 13)

The synergy effects of the antibiotic combinations against the selected isolates were examined by disc diffusion test (Miles, 1996; Mayer and Nagy, 1999) Two discs, each containing one or other of the two tested antibiotics, were placed at a distance of about 20 mm from each other on top of a P. aeruginosa isolate-covered agar plate. Synergy was considered to occur when there was a well-observed change (>2 mm) in the zone of inhibition. The synergy was classified as weak when a change <2 mm was observed in the zone of inhibition (Mayer and Nagy, 1999).

3.15 PLASMID DNA ISOLATION

This procedure was used to extract plasmid DNA from bacterial cell suspensions and was based on the alkaline lysis procedure developed by Birnboim and Doly (Nucleic Acids Research 7:1513, 1979).

1. Sterile 1.5 mL micro centrifuge tubes were labeled and 1000 uL of the

cell suspension was pipetted out from fresh culture into each tube.

2. The caps were closed and the tubes were centrifuged at maximum speed for 20 s.
3. The supernatant was discarded using a micropipette without disturbing the cell pellet.
4. Then 100 uL of Buffer 1 was added to each tube and the cells were resuspended.
5. Then 200 uL of Buffer 2 was added to each tube and the solutions were mixed by rapidly inverting the tubes for few times.
6. Following this the tubes were kept in ice for 5 minutes.
7. Then 150 uL of ice-cold Buffer 3 was added to each tube. The caps were closed and the solutions were mixed by rapidly inverting the tubes for few times. A white precipitate was formed.
8. The tubes were kept in ice for 5 minutes
9. Then tubes were centrifuged at a maximum speed for 5 minutes. After the centrifugation, the supernatants were transferred into clean 1.5 mL tubes, without disturbing the precipitate. The pellet was discarded and the supernatants were kept separately.
10. To each tube of supernatant an equal volume (about 400 μ L) of isopropanol was added for precipitating the nucleic acids. The caps were closed and the solutions mixed vigorously. The tubes were kept at room temperature for 2 minutes and centrifuged at maximum speed for 5 minutes.
11. Then 200 μ L of absolute ethanol was added to each tube and mixed

thoroughly.

12. Then the tubes were centrifuged at a maximum speed for 2-3 minutes.
13. The supernatant was carefully removed and discarded. The tubes were placed in the fume hood with the caps open for 15-20 minutes to dry off the last traces of ethanol.
14. After drying the ethanol 20 μ L of TE buffer was added and the pellet was dissolved.
15. In the end, tubes were labeled and stored it in the freezer for plasmid profile.

REAGENT BUFFER 1:

50 mM Tris-HCl	-	1.576 g
50mMEDTA	-	3.7224 g
100 ug/mL RNase A	-	5 uL
Distilled water	-	200 ml

pH8.0

BUFFER 2:

1% SDS	-	2.0 g
0.2 M NaOH	-	1.6 g
Distilled water	-	200 ml

BUFFER 3:

3.0 M Potassium Acetate - 16.78 g

pH 5.5

18 ml of acetic acid was made upto 54 ml with distilled water. Discarded

11 ml. To remaining 43 ml, 57 ml of 0.3M potassium was added.

3.16 AGAROSE GEL ELECTROPHORESIS (Plate 14)

1. To resolve the plasmid DNA, agarose gel of 0.8% concentration was used.
2. 0 ml 1 Ox TAE buffer was mixed with 90ml of distilled and then 0.4g of agarose was added.
3. The solution was boiled and cooled. Then 10 μ l (10mg/ml)'stock Ethidium bromide was added with the 100 ml agarose solution When it came down to bearable heat, it was poured into the blocks which had suitable comb fixed to it.
4. After the polymerization, the comb was removed.
5. The gel was placed inside the Electrophoretic tank and the buffer was added into it.
6. 30 μ L of the sample was mixed with 5 μ L of loading buffer and loaded in to the wells.

7. The voltage was set at 100 milli Ampere.

When the dye was near end of the gel, the gel was removed.

8. The gel was read for plasmids and documented under UV Trans illumination with the help of gel documentation system.

REAGENT

10X TAE BUFFER

Tris base - 48.4 gram

Glacial acetic acid - 11.42 ml

0,5 M EDTA - 20 ml

pH - 8.0

LOADING DYE (6X):

40% sucrose

0.01 mg of Bromophenol Blue powder

Distilled water

MARKERS

λ DNA - HIND - III and (£>x 174. DNA Hae III digests were used as marker DNA

Results

During the study period from October 2005 to September 2006 a total of 110 Ear Swabs were collected from 100 Patients with CSOM attending ENT op Coimbatore Medical College hospital and Ear swabs were processed in the laboratory of microbiology department Coimbatore Medical College Hospital Coimbatore. Out of 100 patients with CSOM 10 patients were suffering from bilateral CSOM (Fig.1). About 114 Organisms were isolated from 110 specimens. Among 110 specimens 105 numbers showed positive cultures (95.45%) This study showed that most common isolates obtained from CSOM patients were pseudomonas aeruginosa (37.7%), Staphylococcus aureus (32.4%), Proteus sp (12.2%), Klebsiella sp (7.8%), Coagulate negative staphylococcus aureus (2.6%), Streptococcus pyogens (1.7%), E. Coli (0.8%), Moraxella catarrhalis (0.8%), Candida Sp (2.6%), Aspergillus (0.8%). In total of 105 positive cultures 9 cultures showed mixed growth (Table 1).

The Organisms in the mixed growth were pseudomonas aeruginoasa and staphylococcus aureus in 3 cultures, pseudomonas aeruginosa and proteus in 2 cultures, pseudomonas aeruginosa and aspergillus in 1 culture, staphylococcus aureus and Candida species in 3 cultures. Fungal growth was seen in 3.4 % of total isolation. Pseudomonas aeruginosa grew in 43 cultures. The antibiogram patterns showed 23 isolates of pseudomonas aeruginosa which were multi drug resistant (Table 7, Plate 9) (ie resistant to more than one antibiotic) (53.48%), Staphylococcus aureus (32.43%) Klebsiella Sp (33.37) and

TABLE - 1

Organisms Isolated in CSOM

S.No	Name of the Organism	No of isolated	Percentage
1.	Pseudomonas aeruginosa	47	37.7%
2.	Staphylococcus aureus	37	32.4%
3.	Proteus Sp	14	12.2%
4.	Klebsiella Sp	9	7.8%
5.	Coagulase Negative Staphylococcus aureus	3	2.6%
6.	Escherichia coli	1	0.8%
7.	Streptococcus pyogenes	2	1.7%
8.	Moraxella Catarrhal is	1	0.8%
9.	Candida Sp	3	2.6%
10.	Aspergillous Sp	1	0.8

TABLE - 2

Age and Sex wise distribution of isolates in CSOM

Age	Sex	
	Male	Female
1-12 (Paediatric)	14	5
13-20	7	9
21-30	10	21
31-40	7	10
41-50	2	4
51-60	3	3
60<	4	1
Total	47	53
Range	2 – 65 years	1 ½ - 63 years
Percentage	47 %	53 %
Mean	28.95	28.01
Median	27	26

proteus species (14.2%) (Fig. 5) Twenty three isolates of pseudomonas aeruginosa were resistant to cephalosporins. The antibiogram (Table 4, Fig. 4) for pseudomonas also showed the sensitive pattern as Gentamicin 46.5%, Amikacin 79.1% Tobramycin 46.5%, Ciprofloxacin 46.5%, ofloxacin 46.5%, Cefotaxime 46.5%, Ceftazidime 88.4%, Cefepime 86%, meropenam 93 piperacillin. 95.31%. Minimum inhibitory concentration was found out for all the 43 strains of pseudomonas aeruginosa for cefotaxime and ceftazidime by Hi Comb (E. Test) method (Table 5) By this method 21 (48.8 %) strains were

found to be resistant to cefotaxime and 5 (11.6) strains were found to be resistant to ceftazidime.

Strains showing MIC value ≥ 16 mg/l were considered resistant for Cefotaxime and ceftazidime (includes intermediate and resistant categories) (Villanova 2000). In the present study 21 Strains showed resistance to Cefotaxim. Out of these 9 (42.85%) strains showed MIC ≥ 64/mg/L and 12 (57.15%) strains showed ranges between 16-32 mg/L for Cefotaxime. When we have tested MIC for Ceftazidime (Total Ceftazidime resistance strain 5 (11.6%), 3 (60%) strains showed MIC ≥ 32 mg/L and 2 (40%) strains showed MIC of ≥ 16/L (Table 8).

Production of inducible β lactamase was detected using double disc diffusion (Disc approximation) (Gencer, etal., 2002; Miles, 1996) test for strains; which were susceptible to cephalosporins. It was observed that 20 (43.5%) isolates were sensitive to cephalosporins with a zone of inhibition falling above 22 mm diameter. Out of these

TABLE - 3

Age and sex wise distribution of pseudomonas aeruginosa

Age	Sex	
	Male	Female
1-12 (Paediatric)	5	1
13-20	3	1
21-30	6	11
31-40	2	6
41-50	1	2
51-60	0	2
60<	2	1
Total	19	24
Range	3- 65 years	11-63 years
Percentage	44.18	54.82
Mean	25.42	33.83
Median	25	29.5

20 strains 8 (40%)were found to produce β- lactamases when induced with a distance of 25mm between two discs (Cefoxitin and Cefotaxime). By reducing the disc distance by 5mm all strains were induced to produce β- lactamases (Table 9).

By using double disc diffusion test, extended spectrum β-lactamases were detected in 23 Strains (Table 8). Which were resistant as per zone of inhibition. When the two discs (Clavulanic acid and cefotaxime) were 25mm apart. Presence of ESBLs was detected in one (4.3%) isolate. When we reduced the distance to 15mm 8 (34.7%) strains were found to produce ESBLs. At 10mm disc approximation, 18 (78.2%) Strains. By this method we found out that 1 (4.35) strain produced only one type of ESBLs and 18 (78.2%) strains produced more than one type

of ESBLs and 4 (17.3%) strains did not produce ESBLs. The mechanism of resistance may be other than production of ESBLs in these strains.

The evaluation of the frequency of the synergic effects of the antibiotic combination was tested by the disc diffusion method. Amikacin and oflaxacin when combined with cefotaxime exhibited an expressed synergic effect (Well characterised change of $\geq$ 2 mm in the in inhibition zone) in 15 (34.9%) and 14 (32.5%) isolates. weak synergic change of $\leq$ 2 mm in the inhibition zone was observed in 6 (13.9%) and 7 (16.3%) isolates. No synergy, no change observed in the inhibition zone (Mayer and Nagy, 1999) in 22 (51.2%) and 22 (51.2%) isolates (Table 10).

TABLE - 4

Antibiotic susceptibility testing by Muller Hinton Agar disc diffusion methods.

S. No	Name of the Antibiotics	No (7) us Sensitive	No (%) of Percentage Resistance
1	Gentamicin	20 (46.5)	23 (53.5)
2	Amikacin	3 (79.1)	9 (20.9)
3	Tobramycin	20 (46.5)	23 (53.5)
4	Ciprofloxacin	20 (46.5)	23 (53.5)
5	Ofloxacin	20 (46.5)	23 (53.5)
6	Cefotaxime	20 (46.5)	23 (53.5)
7	Ceftazidime	38 (88.4)	5 (11.6)
8	Cefepime	37 (86)	6 (14)
9	meropenam	40 (93)	3 (7)
10	Piperaccillin	41 (95.34)	2 (4.66)

TABLE - 5

MICS of Selected cephalosporins against 43-pseudomonas aeruginosa isolates from CSOM

Isolate	MICS of Antibiotics (Break point of Resistance in Mg/L)	
	Cefotaxime (≥ 64)	Ceftazidime ≥ 32)
1	3	0.5
2	64	5
3	16	1
4	7.5	5
5	7.5	1
6	7.5	3
7	240	120
8	16	1
9	32	5
10	32	5
11	5	0.5
12	16	1
13	60	3
14	7.5	0.5
15	32	3
16	32	1
17	30	16
18	7.5	0.5
19	7.5	5
20	16	5

21	120	2
22	32	3
23	7.5	2
24	5	1
25	16	1
26	120	1
27	7.5	5
28	240	10
29	60	16
30	3	0.5
31	5	0.5
32	3	1
33	4	0.5
34	7.5	64
35	7.5	2
36	32	5
37	7.5	32
38	5	0.5
39	60	3
40	3	1
41	7.5	1
42	120	3
43	10	7.5

TABLE - 6

Age wise distribution of multidrug resistance pseudomonas aeruginosa

Age	No of PA isolated	No of MDR PA isolates	Percentage of MDR PA
1-12 (Pediatric)	6	3	50
13-20	4	2	50
21-30	17	8	47.05
31-40	8	3	37.50
41-50	3	2	66.6
51-60	2	2	100
60<	3	3	100
Total	43	23	53.48

TABLE - 7

Comparison of multidrug resistant in different isolates

S. No	Name of the organisms	Total Number of isolates	No of MDR Isolates	Percentage
1.	Pseudomonas aeruginosa	43	20	53.48
2.	Staphylococcus aureus	37	12	32.4
3.	Proteus Sp	14	2	14.3
4.	Klebsiella Sp	9	3	33.3
5.	Cons	3	Nil	0
6.	Streptococcus Pyogens	2	Nil	0
7.	Escherichia coli	1	Nil	0
8.	Moraxella Catarrhalis	1	Nil	0

TABLE - 8

Detection of extended spectrum β - Lactamases by double disc synergy method (Total Resistant Strains 23)

Various disc distances between clavulanic acid and cefotaxime	No (%) of Positive	No. (%) of Negative
25mm	1 (4.4)	22 (95.6)
20 mm	1 (4.4)	22 (95.6)
15 mm	8 (34.7)	15 (65.3)
10 mm	18 (78.3)	5 (21.7)

TABLE - 9

Detection of inducible B – Lactoamases by disc approximation method (Total sensitive strain 20)

Various disc distances between cefoxitin and cefotaxime	No (%) of positive	No (%) of Negative
25mm	8 (40%)	12 (60%)
20mm	20 (100)	0

TABLE - 10

Synergy effects of combinations of Aminoglycosides and fluroquinolones with third generation cephalosporins

Combination	No (%) of effective synergy	No (%) of weak synergy	No (%) of No Synergy
Amikacine + Cefotaxime	15 (34.9)	6 (13.9)	22 (51.2)
Amikacine + Ceftazidime	16 (37.2)	7 (16.3)	20 (46.5)
Ofloxacin + Cefotaxime	14 (32.5)	7 (16.3)	22 (51.2)
Ofloxacine + Ceftazidime	16 (37.2)	6 (13.9)	21 (42.9)

TABLE - 11

Distribution of Pseudomonas isolates in different group of population.

Population	No of Pseudomonas isolated	Percentage.
Agricultural	15	34.8
Construction	8	18.7
Pre School	1	2.4
School	8	18.7
College	2	4.6
Employee	5	11.6
House wife	1	2.4
Others	3	6.9

When the combinations of Amikacin and ofloxacin with Ceftazidime were tested by this method, synergy was observed in 23 (53%) isolates. The epidemiological analysis of the patients revealed the following details. Aerobic bacterial isolates in middle ear infections did not show any sex predilection though the females were marginally more effected (53%) when compared to male 47% (Table 2, Fig. 2). In pseudomonas isolation from middle ear infection was more in females (55.8%) as in the Bacterial isolates from CSOM (Table 3). Common side of the CSOM infection was52% Left side, 38% Right side and 10% in both side.

Chronic suppurative otitis media infections by pseudomonas aeruginosa were seen in all age groups. The frequency of infection varied in different age

group with more cases in 21-40 years age group (Table 6, Fig. 3). The analysis showed bimodal distribution of cases in which lower incidences were noted in 0-20 and 41-50 age group and higher incidences in 21-40and 50-80 age group. Multidrug resistant pseudomonas infection in middle ear was most commonly seen in middle age group and older age group. Most of the patients affected were agricultural workers (34.8%) (Table 11). The other major working group was construction workers 18.7% followed by school children. 18.7%. The other groups were least affected (Pre School, employees, college students, House wives and others).

Resistant plasmid isolation was done in all ESBLs positive strains by alkaline lysis method developed by Birn boim and Doly. It was observed that plasmids were present in all ESBL positive strains.

Discussion

Chronic suppurative otitis media is considered to be major health problem in the developing world with a relatively high morbidity and mortality. The overall prevalence of CSOM in these countries ranges from 5 -10 % (ManiJJ etal : 1987)). About 50% of brain absesses are otogenic in origin with a mortality rate of 50 -75% (Sulla et al 1989). The bacterial pathogens mainly involved are pseudomonas aeruginosa, staphylococcus aureus, proteus sp and klebsiella sp. Infections lead among the causes of deafness and much importance to be given because of the morbidity they cause and also because of the increasing antibiotic resistance among bacterial isolates. It is becoming increasingly difficulty to control chronic suppurative otitis media through antibiotics. Hence a composite study on the mechanisms of antibiotic resistance is mandatory to formulate antibiotic policies for controlling multi drug resistant infections.

Against this background an attempt was made for ESBL production, inducibility of beta lactamases and isolation of plasmids in multidrug resistant pseudomonas aeruginosa isolates from chronic suppurative otitis media. Patients attending ENT OP Coimbatore medical college hospital Coimbatore from October 2005 to September 2006. The importance of study lies in two reasons.

1. Pseudomonas aeruginosa is the commonest gram negative organism isolated from chronic suppurative otitis media .

2. The main mode of resistance of pseudomonas aeruginosa is through production of beta lactamases which is mainly plasmid mediated.

A total of 110 Specimens collected and processed from chronic suppurative otitios media and 114 organisms were isolated. In which 9 patients had more than one organisms, rest of the patients had only one type of organism. Pseudomonas aeruginosa were isolated from 43 patients. Pseudomonas aeruginosa is the commonest organism isolated from chronic suppurative otitis media (Berry S etal: 1996, Samiullah et al: 2005). About 43 (37.7%) pseudomonas aeruginosa were isolated in our study.

Patients from all age groups were included in this study .It was observed that pseudomonas aeruginosa has been isolated from all age groups but increased isolation was seen in the age group of 21-30 (41.8%). This may be due to the population wise they are high in overall population and having the habit of taking bath in ponds, wells, water falls, swimming pools and also having the habits of introducing foreign bodies like match sticks, bird feather and safety pin in the ear.

In our study we noted multidrug resistance is more commonly seen in pseudomonas aeruginosa 23 (53.48%) than staphycococcus aereus 12 (32.4%), Klebsiella sp 3 (33.3%), proteus sp 2 (14.3%) The antibiogram profile pseudomonas aeruginosa have showed high resistance among antibiotics

Gentamicin 53.5% Tobramicin 53.5%, Ciprofloxacin 53.5%, Ofloxacin 53.5%, Cefotaxime 53.5%, and high sensitivity among antibiotics Amikacin 79.1% , Ceftazidime 88.4%, Cefepime 86%, Meropenam 93%, Piperacillin 95.34% By considering Antibiogram profile of pseudomonas aeruginosa it may be suggested the first line of treatment for multidrug resistant pseudomonas aeruginosa is Amikacin or Ceftazidime or Cefepime and Second line of treatment is meropenam or piperacillin, Multidrug resistance pseudomonas aeruginosa infection was most commonly seen in the age group of more than 50 years. This may be due to that immunity to infections will be less when compared to other age group and hence this increased prevalence rate. Similar findings were observed by samiullah et al; 2005.

There is no significant sex predilection observed when the entire study was considered .However when the occupation was analyzed most of the patients were agricultural workers, who account for 38.8% of the study group. Pseudomonas aeruginosa is a widely prevalent ubiquitous organisms in nature hence agricultural workers are commonly affected. The office going and house wife group were the least affecting groups.

In the present study it was observed that 11.6% of strains were resistant to ceftazidime and 53.5% of strains were resistant to cefotaxime. Bouza observed a similar findings in his study where 15% of the strains of pseudomonas aeruginosa were resistant to ceftazidime In the present study, antibiogram showed that 53.5% of the strains were resistant to cefotaxime .Robert had reported that 46% of the pseudomonas aeruginosa strains were resistant to cefotaxime .Resistant to ciprofloxacin was observed for 53.5%

when compared with other Indian studies the resistance is high in our study.

Inducibility of beta lactamases among strains sensitive to cephalosorin was detected using double disc diffusion method as described by Miles(Miles , 1996) The induction of beta lactamases was seen in 40% of isolates by using an approximation of 25 mm disc distances (Qin, et al; 2004; Miles ,1996).This rate is low when compared to the earlier observation made by Mortiz (Mortiz and Carson.,1996) who had demonstrated 68% of induction for cefotaxime using disc approximation method .But we could induce for all 100% strains after decreasing the distance between cefoxitin and cefotaxime by 5 mm.

This inducibility is significant because different beta lactamases are induced at different disc approximations, hence by decreasing the disc distance we may induce different type of betalactamases, there by proving the capacity of individual strains to induce these enzymes for producing drug resistance.

A similar method was followed for detecting the presence of extended spectrum betalactamases among resistant strains. By standard disc approximation method (25mm) we could detect ESBLs in one strain ,but by reducing disc distances we could show the presence of ESBLs in 78.2% of the strains. There are more than 192 types of betalactamases are discovered (Bradford, 2001).These betalactamases were produced at different concentration gradients of antibiotics (Bert et al.,2003) . Some of them are produced even at low level of selective pressure produced by these antibiotics and some (require high antibiotic concentration for producing betalactamases.

By altering the distances we can vary the concentrations and in turn

vary selective pressures of antibiotics to pseudomonas aeruginosa. This may be possible reason for more strains producing ESBLs at reduced disc approximations According to Bert et al, the standard test with 30 mm distance is insufficient to identify most ESBLs produced by pseudomonas sp (Bert et al., 2003). Strains produced only TEM derived enzymes and one PER-1were detected by use of a 30 mm distance .It was necessary to reduce the distance to 20 mm to detect strains that produce VEB -1 ,SHV29 and OXA 18 .Where as strains that produce the other OXA derived ESBLs were identified only at a distance of 10 or15 mm .In the present study it was not possible to detect the types of ESBLs produced, as it may require molecular type methods .By further expanding the scope of this study ,it is possible to typing these ESBLs with the help of molecular methods and identifying the gene responsible for the resistance can be detected.

In this study synergy between cephalosporins and aminoglycosides and cephalosporins and flouroquinolones was detected by disc diffusion method based on Kirby -Bauer' s antibiotic susceptibility testing as done by Mayer and Nagy (Mayer and Nagy, 1999).The synergy effect of ceftazidime with aminoglycoside was seen against 53.5% and the synergic effect of ceftazidime with flouroquinolones was seen against 50.1% of strains. These combinations may be useful in treating the patients with pseudomonas aeruginosa but cephalosporin aminoglycoside combination is found to be more nephrotoxic than drugs used as monotherapeutic agents.

When combination of cefotaxime with aminoglycoside and flouroquinolones were tested against pseudomonas aeruginosa drug synergy

was observed in 48.8 % and 48.8 respectively. Though in this study synergy was detected with disc diffusion test. Measurement of time killing of the bacteria is the most means of assessing existence synergic effect between drugs (Mayer and Nagy).

Resistant plasmid isolation was done in all ESBL possitive strains (23) by alkaline lysis method developed by Birnboim and Doly. It was observed that plasmids were present all ESBL positive strains, which may be indicative of resistant plasmid.

In conclusion pseudomonas aeruginosa can be agreed upon as the most dreaded Gram negative bacteria among isolates from chronic suppurative otitis media The main mode of resistance among these organisms is through beta lactamase and all the strains can be induced to produce beta lactamases . Through this study we detected ESBLs and plasmid among the multi drug resistant strains all the multidrug resistant pseudomonas aeruginosa. Further research on type's structure and genetic basis for production ESBL will definitely be an important improvement, which will surely have a major bearing on treatment. All the ESBL producing strains being sensitive to cephalosporins and sulbactum combination.

This study from October 2005 to September 2006 revealed that 53.5 percent of pseudomonas aeruginosa isolates from CSOM were multidrug resistant. This study showed CSOM infections are commonly due pseudomonas aeruginosa and staphylococcus aureus. How ever pseudomonas aeruginosa is associated with high level of drug resistance to common antimicrobial agents. Ie Gentamicin, Tobramycin ciprofloxacin,

ofloxacin, cefotaxime, (Jacoby GA et al. 1991). Drug resistance in pseudomonas aeruginosa is alarming; therefore, Antimicrobial Agents must be selected according to local sensitivity studies. Enzymes mediated resistance of pseudomonas aeruginosa can be attributed not only to an inducible, chromosomally mediated β –Lactamase that can render broad spectrum antibiotics inactive and it has plasmid mediated β – Lactamase hydrolysis. A major factor that has lead to the increase in antimicrobial resistant strains has been wide spread use of broad spectrum drugs including the use of third generation cephalosporins and fluoroquinolones. The challenge for physician and Surgeon in to develop stricter pathogen surveillance and drug susceptibility testing and to follow Judicious utilisation of both older and newer antimicrobial agents.

Cefepime has greater stability against SHV type enzymes (ESBL) expressed by enterobacteriacae and has greater activity against strain producing these enzymes than other cephalosporins.

Resistance to antimicrobial agents is an increasing public health threat. It limits therapeutic options and leads to increased morbidity and mortality (NNISS data summary 2004) given the increasing rate in pseudomonas aeruginosa, multidrug resistance more prevalent among CSOM patients attending hospitals (or) community. More over the intensity of selection pressure by broad spectrum antibiotics in high in ENT Department. Treatment with multiple antibiotic agents for other resistant organisms and treatment broad spectrum cephalesporins and aminoglycosides specifically also emerged as being important risk factors (Gould I.M. et al; 1994). Infection with MDR pseudomonas aeruginosa in associated with adverse clinical out come,

and strict isolation of patients with MDR micro organisms and Judicial use of antibiotic should be emphasised in order to prevent the spread of MDR pseudomonas aeruginosa. The emergence and spread of antibiotic resistant bacteria and describes research and development aimed at controlling these organisms. It concludes that efforts are necessary both to preserve the effectiveness of currently available antibiotics and develop new antibiotics.

Antibiotic creates “Selective Pressure “that promotes the spread of resistant bacteria. Susceptible bacteria are killed or inhibited and resistant bacteria survive and multiply, as bacteria become resistant to increasing numbers of antibiotics are used more often increasing the selection pressure for bacteria to become resistant to them. The report identified hand washing, improved hygiene and patient isolation as successful infection control efforts.

Finally we can conclude that we should give at the most importance to study the multidrug resistance mechanisms of pseudomonas aeruginosa. Other wise we will face a severe threat from these MDR infectious organisms. So continuous surveillance and research works is mandatory to combat the multidrug resistant infections.

Summary

- 110 Specimens were collected from 100 CSOM patients attending ENT op at Government Medical College Hospital, Coimbatore during October 2005 to September 2006.

- 43 strains of pseudomonas aeruginosa were collected during the study period.

- Antibiotic susceptibility testing was done for these strains by Kirby -Bauer disc diffusion method.

- MIC for all 43 strains were detected using Hicomb test (E-TEST).

- Antibiotic susceptibility of the strains were correlated with their MIC value.

- Inducibility of beta lactamases were detected for the 20 sensitive trains isolated, by double disc diffusion test and it was observed that all 20 strains were induced to produce betalactamases with disc approximation at 20 mm apart. Only 8(40%) were induced to produce betalactamases when the discs were 25 mm apart.

- ESBLs were detected for the resistance strains by double disc diffusion test. Presence of ESBL was detected in 4.3 % strains through 20mm disc approximation, 34.7% of strains through 15mm of disc approximation, 78.2% through 10 mm disc approximation.

- The synergic effect between

  Cephalosporins and aminoglycoside

  Cephalosporins and flouroquinolones

Were detected using double disc diffusion method .It was observed that synergy between aminoglycoside and cephalosporins was found to be maximum 53.5% :

- Plasmid profile analysis was done for all ESBL positive strains and plasmids were detected in all ESBL positive strains.

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Master Chart

MASTER CHART – ABBREVIATION

PA	–	Pseudomonas Aeruginosa
Kle	–	Klebsiella sp
Sa	–	Staphylococcus aureuss
Pro	–	Proteus Sp
Str	-	Streptococcus pyogens
Es. Coli	-	Escherichia Coli
Mx	-	Moraxella Catarrhalis
Cons	-	Coagulase negative staphylococcus aureus
A	-	Asperigillus sp
C	-	Candida sp

Occuption

1. Agri
2. Construction worker
3. Pre – School
4. School
5. College
6. Employee
7. House wife
8. Others

Oc	-	Occupation
CF	-	Clinical Features
ED	-	Ear Discharge
HoH	-	Hard of hearing
URTI	-	upper Respiratory Tract Infection
CSOM	-	Chronic Suppurative Otitis Media
GNB	-	Gram Negative Bacilli
GPC	-	Gram Positive Cocci
O	-	Other organisms
SDA	-	Sabouraud;s Dextrose Agar
BAP	-	Blood Agar Plate
CET	-	Cetrimide Agar
CA	-	Choclate Agar

MAC	-	Macconkey Agar
M	-	Male
F	-	Female
MOT	-	Motility test
NA42/Pig	-	Growth at 42 ºC in Nutrient Agar/Pigmentation.
NLF	-	Non Lactose Fermenter.
LF	-	Lactose Fermenter
MLF	-	Minute Lactose Fermenter
LFM	-	Lactose Fermenter mucoid
Mix	-	Mixed
C	-	Catalase
OX	-	Oxidase
SFT	-	Sugar Fermentation Test
OFT	-	Oxidation. Fermentation Test
AK	-	Amikacin
CIP	-	Ciprofloxacin
CEF	-	Cefotaxime
CTZ	-	Ceftazidime
MERO	-	Meropenam
MDR	-	Multidrug resistant
SYN	-	Synergic Test
MIC	-	Minimum Inhibitory concentration
IND β L	-	Inducible β – Lactamase
ESBL	-	Extend Spectrum of β – Lactamase.
S	-	Sensitive
R	-	Resistant
E	-	Effective
W	-	Weak
AST	-	Antibiotic Sensitivity test.

MASTERCHART

SNO	NAME	AGE	SEX	OPNO	OC	CF			CSOM	GRAMS			BAP/CA			MAC	SDA	CET	NA42/Pig	MCT	C	O	SFT	OFT	ISOLATES	AST					MDR	SYN	MIC		INDβ-L	ESBL	PLASMID
						ED	HOH	URTI		GNB	GPC	O	GNB	GPC	O											AK	CIP	CEF	CTZ	MERO			CEF	CTZ			
1.	Veeramuthu	19	M	25737	8	+	-	+	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	3	0.5	+		
2.	Rajendiran	40	M	25905	8	+	+	-	Rt	+	-	-	+	-	-	NLF	-	-	-	+	+	-	+	F	Pro	S	S	S	S	S	-						
3.	Manjula	11	F	25901	4	+	+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+	E	64	5		+	+
4.	Sadam Husain	12	M	25954	4	+	-	+	Lt	+	-	-	+	-	-	LF	-	-	-	-	+	-	+	F	Kle	S	S	S	S	S	-						
5.	Rahim	29	M	26330	1	+	+	-	Rt	+	-		+			NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+	W	16	1		+	+
									Lt	-	-	-	-	+	-	NLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
6.	Rajendren	34	M	26623	2	+	+	-	Lt	+	-	-	+	-	-	MLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	7.5	5	+		
7.	Sahisudheen	28	M	26730	1	+	-	+	Rt	+	-	-	+	-	-	NLF	-	-	-	-	+	-	+	F	Kle	S	S	S	S	S					+		
									Lt	+	-	-	+	-	-	LFM	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	W	7.5	5			
8.	Renganathan	64	M	27113	8	+	+	-	Rt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NG											
9.	Krishnan	53	M	27180	2	+	+	-	Lt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NG											
10.	Balamurugan	29	M	27572	5	+	-	+	Lt	-	-	+	-	+	+	MLF	+	-	-	-	+	-	+	F	SA+c	S	R	R	S	S	+						
11.	Ramakrishnan	30	M	27255	5	+	+	-	Rt	-	-	-	-	+	-	-	-	-	-	-	-	-	+	F	Str	S	S	S	S	S	-						
12.	Chinnasamy	37	M	27556	1	+	+	-	Lt	-	-	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
13.	Prakash	35	M	27679	2	+	+	+	Lt	+	-	-	+	+	-	M	-	-	-	+	+	+	-	O	PA	S	S	S	S	S	-	E	7.5	3	+		
																				-	+	-	+	F	SA	S	S	S	S	S	-						
14.	Priyadarshini	1½	F	28080	3	+	+	-	Rt	-	-	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
15.	Kerjim	32	M	28076	8	+	+	+	Rt	+	-	-	+	-	-	NLF	-	-	-	+	+	-	+	F	Pro	S	S	S	S	S	-						
16.	Palamvel	20	M	28127	5	+	-	+	Lt	-	-	-	+	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
17.	Shreya	23	F	28153	5	+	+	+	Lt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NG											
18.	Manickammal	60	F	28003	2	+	-	+	Rt	+	-	-	-	-	-	NLF	AS	+	+	+	+	+	-	O	PA+AS	R	R	R	R	R	+	W	240	120		+	+
19.	Senthil Kumar	30	M	28244	1	+	+	+	Rt	-	-	-	+	+	-	-	-	-	-	-	-	-	+	F	Cons	S	S	S	S	S	-						
						+			Lt	-	-	-	+	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
20.	ISSAN	4	M	28267	4	+	+	-	Rt	+	-	-	+	+	-	Mix	-	-	+	+	+	+	-	O	PA	S	R	R	S	S	+		10	1		+	+
																	-	-	-	-	+	-	+	F	SA	R	R	R	R	R	+						

SNO	NAME	AGE	SEX	OPNO	OC	CF			CSOM	GRAMS			BAP/CA			MAC	SDA	CET	NA42/Pig	MCT	C	O	SFT	OFT	ISOLATES	AST					MDR	SYN	MIC		INDβ-L	ESBL	PLASMID
						ED	HOH	URTI		GNB	GPC	O	GNB	GPC	O											AK	CIP	CEF	CTZ	MERO			CEF	CTZ			
21.	Subramani	44	M	28285	5	+	+	-	Lt	+	-	-	+	-	-	NlF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	7.5	1	+		
22.	Babu	46	M	28288	8	+	-	-	Rt	-	+	-	+	-	-	NLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
23.	Priya	20	F	28431	4	+	+	+	Lt	+	-	-	+	+	-	MLF	-	-	-	-	+	-	+	F	Pro	S	S	S	S	S	-						
24.	Ahamedshan	63	M	28364	2	+	-	+	Lt	-	+	-	-	-	+	NLF	+	-	-	-	+	-	+	F	SA+c	S	R	R	S	S	-						
25.	Azhandheen	11	M	28419	4	+	+	-	Rt	-	+	-	-	-	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	+						
26.	Manikandan	25	M	28444	1	+	+	+	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+		3.2	5	-	+	+
27.	Kumarasamy	32	M	28365	2	+	-	+	Lt	+	-	-	+	-	-	LFM	-	-	-	-	+	-	+	F	Kle	S	S	S	S	S	-						
28.	Praveen kumar	13	M	28571	4	+	+	-	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	5	0.5	+		
29.	Viapuri	65	M	28666	8	+	+	-	Lt	+	-	-	+	-	-	NLF	-	-	+	+	+	+	-	O	PA	R	R	R	S	S	+		16	1		+	+
30.	Ravikumar	23	M	28700	5	+	+	-	Lt	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	NG												
31.	Karuppasamy	54	M	19382	2	+	+	-	Rt	-	+	-	-	-	-	MLF	-	+	-	-	+	-	+	F	SA	S	S	S	S	S							
32.	Subhiksha	42	F	18990	7	+	+	-	Rt	+	-	+	-	+	-	LF	-	-	-	-	+	-	+	F	Kle	S	R	R	S	S	+						
33.	Paridha	22	F	18616	5	+	-	+	Rt	+	-	-	-	+	-	NLF	-	-	+	+	+	+	-	O	PA	S	R	R	S	S	+	W	60	3		+	+
34.	Emilini	23	F	18660	5	+	+	-	Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
35.	Lakshmi	27	F	18413	6	+	+	-	Lt	-	+	-	+	+	-	MLF	-	+	-	-	+	-	+	F	SA	S	S	S	S	S	-	E	7.5	3	+		
36.	Karthikeyan	17	M	17700	5	+	+	-	Lt	-	+	-	+	-	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S					+		
37.	Amsagiri	8	M	17582	1	+	+	-	Rt	+	-	-	-	+	-	NLF	-	-	+	+	+	+	-	O	PA	S	S	S	S	S	-						
38.	Myrose	56	F	17339	4	+	-	+	Rt	+	-	-	+	-	-	LFM	-	-	-	-	+	-	+	F	Kle	S	S	S	S	S	-						
39.	Santhosh	45	M	17585	4	+	+	-	Lt	-	+	-	+	+	-	MLF	-	+	-	-	+	-	+	F	SA	S	S	S	S	S	-						
40.	Ramani	17	M	17373	8	+	+	-	Rt	+	-	-	-	-	-	Mix	-	-	-	-	+	-	+	F	SA+c	S	S	S	S	S	-						
						+			Lt	-	+	-	-	+	-		-	-	-	-	-	-	+	F	Cons	S	S	S	S	S	-						

SNO	NAME	AGE	SEX	OPNO	OC	CF			CSOM	GRAMS			BAP/CA			MAC	SDA	CET	NA42/Pig	MCT	C	O	SFT	OFT	ISOLATES	AST					MDR	SYN	MIC		INDβ-L	ESBL	PLASMID
						ED	HOH	URTI		GNB	GPC	O	GNB	GPC	O											AK	CIP	CEF	CTZ	MERO			CEF	CTZ			
41.	Bhackiyam	16	M	17417	6	+	+	-	Lt	+	-	-	-	-	-	NLF	-	-	+	+	+	+	-	O	PA	R	R	R	R	R	+	W	240	120		+	+
42.	Vijayalakshmi	13	F	16776	4	+	-	-	Lt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NG	S	S	S	S	S	-						
43.	Nithya	26	F	16502	4	+	+	-	Lt	+	-	-	+	+	-	NLF	-	+	-	+	+	-	+	F	Pro	S	S	S	S	S	-						
44.	Nandhini	35	F	16520	4	+	-	-	Rt	+	-	-	+	+	-	NLF	-	-	-	+	+	-	+	F	Pro	S	R	R	S	S	+						
45.	Gowri	42	F	16379	1	+	+	-	Lt	+	-	-	-	+	-	NLF	-	-	+	+	+	+	-	O	PA	S	R	R	S	S	+		32	1		+	+
									Rt	-	-	-	-	+	-		-	-		+	+	-	+	F	SA	S	S	S	S	S	-						
46.	Pappammal	24	F	2856	2	+	+	+	Rt	+	-	-	+	+	-	NLF	-	+	+	+	+	+	-	O	PA	R	R	R	R	R	+		32	16		+	+
47.	Gandhi mathi	35	F	7939	1	+	+	-	Rt	-	-	-	+	-	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S							
48.	Pappathy	35	F	19955	1	+	+	-	Rt	+	-	-	-	+	-	NLF	-	-	+	+	+	+	+-	O	PA	S	S	S	S	S	-						
49.	Sasikala	28	M	20682	1	+	+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-		7.5	5	+		
50.	Dhanalakshmi	63	F	12114	7	+	+	-	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+		16	5		+	+
51.	Gopinath	8	M	20694	4	+	-	+	Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
52.	Sundari	40	F	22667	1	+	-	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+		120	2		+	+
53.	Gopalkrishnan	63	M	85472	8	+	+	-	Rt	+	-	-	+	-	-	NLF	-	-	+	+	+	+	-	O	PA	R	R	R	S	S	+		32	3		+	+
									Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	R	R	R	R	R	+							
54.	Santhi	28	F	2599	7	+	+	-	Lt	-	-	-	-	-	-	-	-	-	-	-	-	-	+	F	CONS	S	S	S	S	S	-						
55.	Britto	3	M	28419	3	+	-	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	7.5	2	+		
									Lt	-	-	+	-	-	+										Mx	S	S	S	S	S	-						
56.	Afna	4	F	29731	3	+	-	-	Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
57.	Vinoth	9	M	1679	4	+	+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	W	5	1	+		
									Lt	+	-	-	+	-	-	NLF	-	-	-	+	+	-	+	F	PRO	S	S	S	S	S	-						
58.	Veerammal	29	F	1738	1	+	-	+	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+		16	1		+	+
59.	Kalai vani	32	F	1784	6	+	-	+	Lt	-	+	-	-	+	-	MLF	+	-	-	-	+	-	+	F	SA+c	R	R	S	S	S	+						
60.	kannan	10	M	1786	4	+	-	-	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+		32	1		+	+

SNO	NAME	AGE	SEX	OPNO	OC	CF			CSOM	GRAMS			BAP/CA			MAC	SDA	CET	NA42/Pig	MCT	C	O	SFT	OFT	ISOLATES	AST					MDR	SYSN	MIC		IND β-L		PPASMID
						ED	HOH	URTI		GNB	GPC	O	GNB	GPC	O											AK	CIP	CEF	CTZ	MERO			CEF	CTZ			
61.	Malaevili	5	F	36745	4		+	-	Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	R	R	R	S	+						
62.	Sundharapandi	30	M	37192	6		+	-	Rt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
									Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-		7.5	5	+		
63.	Dowlath	34	F	39318	7		-	-	Rt	+	-	-	+	-	-	NLF	-	-	-	+	+	-	+	F	PRO	S	S	S	S	S	-						
64.	Banu	16	F	2041	4		-	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	R	R	S	S	+		240	10		+	+
									Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	R	R	S	S	+						
65.	Kavitha	25	F	2304	1		+	-	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	R	R	R	R	R	+		60	16		+	+
66.	Ranju	40	F	2524	2		+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	W	3	0.5	+	-	
67.	Jeyanthi	31	F	2557	6		-	+	Rt	-	+	-	-	+	-	MLF	-	-	-	-	-	-	+	F	SA	S	S	S	S	S	-						
68.	Backiyam	45	F	2689	6		-	+	Lt	+	-	-	+	-	-	NLF	-	-	-	+	+	-	+	F	PRO	S	S	S	S	S	-						
69.	Chandra	20	F	2296	5	+	-	-	Lt	+	-	-	+	-	-	LFM	-	-	-	-	+	-	+	F	Kle	S	S	S	S	S	-						
70.	Dhanasekar	14	M	2813	4		+	-	Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	R	R	S	S	+						
71.	Duraiammal	26	F	3063	1	+	+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	5	0.5	+		
72.	Meghala	25	F	3072	5		-	+	Lt	+	-	-	+	-	-	NLF	-	-	-	+	+	-	+	F	PRO	S	S	S	S	S	-						
73.	Parameswari	25	F	3202	7	+	+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	4	0.5	+		
74.	Kannammal	27	F	3211	6		+	-	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	F	PA	S	S	S	S	S	-	E	3	1	+		
75.	Lakshmi	25	F	3242	6		-	-	Lt	+	-	-	+	-	-	LFM	-	-	-	-	+	-	+	F	Kle	R	R	R	R	R	+						
76.	Fathima	29	F	3696	1		-	-	Lt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	R	S	S	R	S	+		7.5	60		+	+
77.	Marimuthu	4	M	4332	3		-	-	Lt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
78.	Prasanna	8	M	4430	4		+	-	Rt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	S	S	S	S	-						
79.	Jaya	24	F	4485	5		-	+	Rt	+	-	-	+	-	-	NLF	-	+	+	+	+	+	-	O	PA	S	S	S	S	S	-	E	7.5	2	+		
80.	Mangattha	37	F	4492	6		+	-	Rt	-	+	-	-	+	-	MLF	-	-	-	-	+	-	+	F	SA	S	R	R	R	S	+						

SNO		AGE	SEX	OPNO	OC	CF	CSOM	GRAMS	BAP/CA	MAC	SDA	CET	NA42/Pig	MCT	C	O	SFT	OFT	ISOLATES	AST	MDR	SYN	MIC	IND	ESBL	PLASMI